BuMP-ing up Insulin Secretion by Pancreatic β Cells

Maureen Gannon[1,*]
[1] Department of Medicine, Division of Diabetes, Endocrinology, and Metabolism, Vanderbilt University Medical Center, Nashville, TN 37232, USA
*Correspondence: maureen.gannon@vanderbilt.edu
DOI 10.1016/j.cmet.2007.02.003

While several transcription factors are known to increase insulin production and secretion, their therapeutic potential for treatment of type 2 diabetes remains unrealized. In this issue, Goulley et al. (2007) show that BMP signaling specifically regulates genes involved in insulin production and secretion and demonstrate that exogenous BMP4 administration augments glucose-stimulated insulin secretion in vivo.

Type 2 diabetes results from combined insulin resistance in peripheral insulin-responsive tissues (liver, muscle, and fat) and decreased functional β cell mass. In normal β cells, glucose metabolism leads to increased intracellular ATP, closure of ATP-dependent K^+ channels, membrane depolarization, and Ca^{2+} influx, resulting in insulin granule exocytosis (Figure 1A). The β cells of individuals with type 2 diabetes exhibit decreased glucose-stimulated insulin secretion (GSIS). Several transcription factors are known to regulate insulin gene expression and mature β cell function (Jensen, 2004). However, much less is known about the secreted signals regulating GSIS. Therapeutically, administration of a circulating factor to enhance insulin secretion is much more tangible than trying to increase expression or activity of a β cell transcription factor. Insulin itself acts in an autocrine manner to stimulate further insulin production and secretion (Otani et al., 2004). In addition, the incretin hormones glucagon-like polypeptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) act in a paracrine manner to enhance GSIS following a meal. GLP-1 also has long-term effects on the β cell, including increased insulin gene transcription through activation of the IPF1/PDX1 transcription factor, increased β cell proliferation, and increased β cell survival (Nielsen et al., 2001). Indeed, GLP-1 analogs are currently in use in the treatment of type 2 diabetes. In this issue of *Cell Metabolism*, Goulley et al. (2007) provide strong evidence that bone morphogenetic proteins (BMPs) should be added to the list of secreted factors that stimulate insulin production and secretion.

Members of the transforming growth factor β (TGF-β) superfamily of growth factors include TGF-β itself, activins, and BMP. TGF-β, activin, and their receptors have been shown, through gene inactivation and transgenic overexpression studies, to play a role in islet morphogenesis and establishment of β cell mass (Kim et al., 2000; Sanvito et al., 1994; Smart et al., 2006; Yamaoka et al., 1998). In general, inhibition of TGF-β signaling results in decreased islet mass, increased numbers of α cells, and poorly organized islets, while enhanced TGF-β signaling results in increased endocrine mass at the expense of the exocrine pancreas. In adult islets, TGF-β has been shown to enhance insulin production and secretion. Loss of activin receptor signaling causes a severe reduction in insulin and glucagon expression and a marked reduction in the size and number of islets. To date, BMP signaling has been implicated only in very early aspects of pancreas specification in zebrafish and chick and in induction of insulin gene expression in cell lines (Song et al., 2006; Yew et al., 2005). Prior to the present study by Goulley et al. (2007), it was unknown whether BMP signaling plays a role in β cell mass regulation or mature β cell function.

Goulley et al. (2007) found that BMPR1A (also known as ALK3) and its high-affinity ligand BMP4 were expressed throughout the pancreatic epithelium at embryonic day (E) 13 but were restricted to endocrine clusters in E15 embryos or islets in neonates. Expression was also detected by RT-PCR in adult islets from mice and humans. BMP4 was the only BMPR1A ligand expressed in islet endocrine cells beginning at the secondary transition (E13.5–E18.5), the period during which the majority of cells that will give rise to mature adult islets are generated. No colabeling studies were performed, so it is unclear whether BMP4 and its receptor are expressed only in β cells; however, these expression analyses suggest that an autocrine BMP signaling pathway is operative in at least the β cell population (Figure 1B). The authors then examined the role of BMP signaling in pancreas development and mature β cell function by generating and characterizing multiple complementary transgenic and knockout mouse lines in which BMP signaling was either inhibited or augmented in the mouse pancreas. Since null mutations in *Bmpr1a* result in early embryonic lethality (Mishina et al., 1995), the authors used a floxed allele of *Bmpr1a* (Ming Kwan et al., 2004) to inactivate the gene specifically in β cells using the rat insulin promoter to drive expression of Cre recombinase (*Ins-Cre*). Alternative strategies to interfere with BMP signaling included the use of the *Ipf1/Pdx1* promoter to express cDNAs for a dominant-negative (dn) *Bmpr1a*, the extracellular BMP

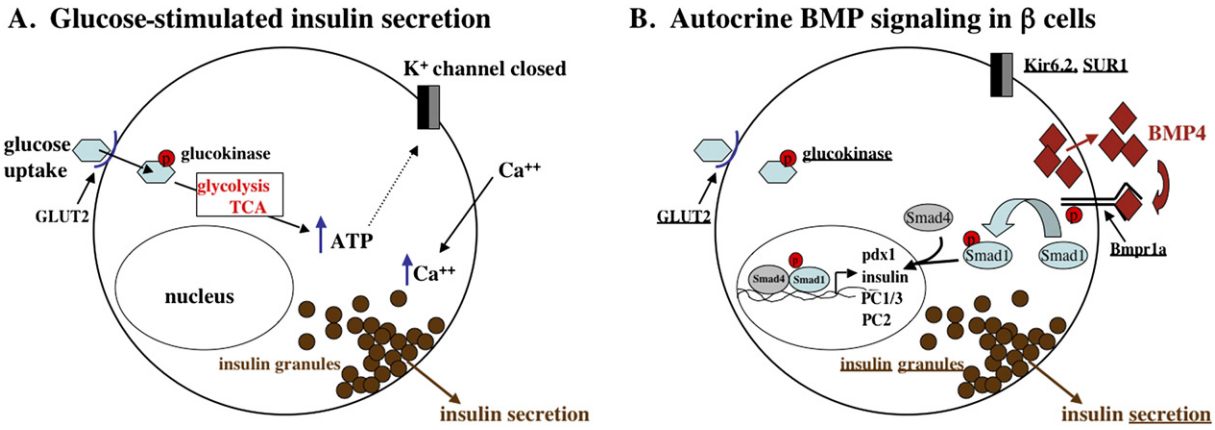

Figure 1. BMP Signaling Affects Insulin Production and Secretion in Pancreatic β Cells

(A) Glucose is taken up by the β cell through the membrane-bound GLUT2 transporter. In the cytoplasm, glucose is phosphorylated by glucokinase to initiate glycolysis. ATP produced during glucose metabolism binds to and inactivates the plasma membrane potassium channel, thus allowing an inward flow of Ca^{2+} ions. This influx of Ca^{2+} causes the insulin-containing vesicles to fuse with the plasma membrane, releasing insulin into the bloodstream.

(B) β cells produce and secrete BMP4, which binds to a heteromeric type 1 and type 2 receptor, resulting in phosphorylation of the type 1 receptor and its downstream second messenger, Smad1/5/8. Phosphorylated Smad1 dimerizes with the ubiquitous Smad4 and translocates to the nucleus to mediate transcription of downstream target genes. In the β cell, these include genes involved in insulin transcription and processing (shown in the nucleus) as well as *Bmpr1a* itself and genes important for glucose sensing, secretion coupling, granule fusion, and insulin secretion (underlined).

inhibitor *noggin*, and the intracellular inhibitor of BMP signaling *Smad6* throughout the developing pancreas.

Animals lacking BMPR1A in β cells were healthy and indistinguishable from control littermates until 2–3 months of age, at which time they showed an impaired ability to clear a glucose load from the bloodstream during an intraperitoneal glucose tolerance test. These mice eventually progressed to overt diabetes by about 6 months of age. Total endocrine mass and α:β cell ratio were unchanged in *Bmpr1a* mutants, and thus, the authors conclude that BMP signaling in the pancreas is not required for islet/β cell development. This group has previously shown that this *Ins-Cre* line of mice exhibits significant recombination in β cells only after weaning (3–5 weeks after birth) (Ahlgren et al., 1998). It is unclear in the present study whether the *Bmpr1a* gene is completely inactivated embryonically or only in adult islets; thus, taken alone, these data would be insufficient proof for a lack of requirement of BMP4/BMPR1A signaling in islet β cell development or a specific role in maintenance of postnatal β cell function. However, the elegance of the study lies in the multiple approaches used to interfere with BMP4/BMPR1A signaling. Overexpression of *dnBmpr1a*, *noggin*, or *Smad6* in the pancreas results in the same phenotype as β cell-specific *Bmpr1a* inactivation. The diabetes associated with loss of BMP signaling was characterized by a dramatic decrease in GSIS as well as loss of responsiveness to other nonglucose secretagogues. Therefore, unlike TGF-β or activin signaling, BMP4/BMPR1A signaling is not required for establishing β cell mass or islet morphogenesis but instead seems to be required only for maintenance of mature β cell function.

Quantitative RT-PCR revealed that reduced BMP signaling decreased expression of *Bmpr1a* itself, suggesting a positive feedback loop similar to that described for signaling by insulin through its receptor in the β cell. In addition, expression of genes important for several aspects of GSIS was significantly reduced in the absence of BMPR1A signaling, including genes involved in glucose sensing and glucose metabolism (*Glut2* and glucokinase [*GCK*]), secretion coupling (*Kir6.2* and *SUR1*), insulin expression and processing (*Ipf1/Pdx1*, insulin [*Ins*], *PC1/3*, and *PC2*), and insulin granule exocytosis (*Rab3d*, *Rab27a*, *calpain-10*, and *SNAP-25*). Interestingly, several of these BMP targets (*Ipf1/Pdx1*, *GCK*, and *calpain-10*) are known susceptibility genes for type 2 diabetes.

In case the multiple experiments to inactivate BMPR1A signaling were not convincing enough, the authors also performed the converse experiment by overexpressing *Bmp4* under the control of the *Ipf1/Pdx1* promoter and again found no alterations in pancreas development or β cell mass, but rather a specific enhancement of postnatal β cell function and improved GSIS. Genes found to be downregulated in the absence of BMPR1A signaling were upregulated in the face of increased BMP4, strongly suggesting that these genes are coordinately regulated in response to autocrine BMP signaling in the β cell (Figure 1B). The authors pave the way for future therapeutic potential of BMP to augment GSIS by showing that injection of recombinant BMP4 stimulates insulin secretion and improves glucose tolerance in wild-type mice without causing hypoglycemia—i.e., the effects of BMP4 are glucose dependent—and, furthermore, that BMP4 can ameliorate the impaired glucose tolerance present in *Ipf1/Pdx1* heterozygous mutant mice.

The identification of extracellular signals that stimulate GSIS provides great therapeutic potential for patients with type 2 diabetes. The specificity of

BMP4 in this process awaits the β cell-specific inactivation of the *Bmp4* gene, a floxed allele of which already exists. However, as mentioned above, both *Bmp4* and *Bmpr1a* are expressed in adult human islets, suggesting that this pathway is operational in humans and thus may be a target for enhancing insulin secretion in man.

REFERENCES

Ahlgren, U., Jonsson, J., Jonsson, L., Simu, K., and Edlund, H. (1998). Genes Dev. *12*, 1763–1768.

Goulley, J., Dahl, U., Baeza, N., Mishina, Y., and Edlund, H. (2007). Cell Metab. *5*, this issue, 207–219.

Jensen, J. (2004). Dev. Dyn. *229*, 176–200.

Kim, S.K., Hebrok, M., Li, E., Oh, S.P., Schrewe, H., Harmon, E.B., Lee, J.S., and Melton, D.A. (2000). Genes Dev. *14*, 1866–1871.

Ming Kwan, K., Li, A.G., Wang, X.J., Wurst, W., and Behringer, R.R. (2004). Genesis *39*, 10–25.

Mishina, Y., Suzuki, A., Ueno, N., and Behringer, R.R. (1995). Genes Dev. *9*, 3027–3037.

Nielsen, J.H., Galsgaard, E.D., Moldrup, A., Friedrichsen, B.N., Billestrup, N., Hansen, J.A., Lee, Y.C., and Carlsson, C. (2001). Diabetes *50*, S25–S29.

Otani, K., Kulkarni, R.N., Baldwin, A.C., Krutzfeldt, J., Ueki, K., Stoffel, M., Kahn, C.R., and Polonsky, K.S. (2004). Am. J. Physiol. Endocrinol. Metab. *286*, E41–E49.

Sanvito, F., Herrera, P.L., Huarte, J., Nichols, A., Montesano, R., Orci, L., and Vassali, J.D. (1994). Development *120*, 3451–3462.

Smart, N.G., Apelqvist, A.A., Gu, X., Harmon, E.B., Topper, J.N., MacDonald, R.J., and Kim, S.K. (2006). PLoS Biol. *4*, e39.

Song, J., Kim, H.J., Gong, Z., Liu, N.A., and Lin, S. (2006). Dev. Biol., in press. Published online December 1, 2006. 10.1016/j.ydbio.2006.11.040.

Yamaoka, T., Idehara, C., Yano, M., Matsushita, T., Yamada, T., Ii, S., Moritani, M., Hata, J., Sugino, H., Noji, S., and Itakura, M. (1998). J. Clin. Invest. *102*, 294–301.

Yew, K.H., Hembree, M., Prasadan, K., Preuett, B., McFall, C., Benjes, C., Crowley, A., Sharp, S., Tulachan, S., Mehta, S., et al. (2005). J. Biol. Chem. *280*, 32209–32217.

Sweet Dreams for LXR

Mitchell A. Lazar[1,*] and Timothy M. Willson[2,*]
[1] Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, The Institute for Diabetes, Obesity, and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA
[2] Discovery Medicinal Chemistry, Molecular Discovery Research, GlaxoSmithKline, Research Triangle Park, NC 27709, USA
*Correspondence: lazar@mail.med.upenn.edu (M.A.L.), tim.m.willson@gsk.com (T.M.W.)
DOI 10.1016/j.cmet.2007.02.001

Nuclear receptors (NRs) are transcription factors whose activities are modulated by the binding of small lipophilic ligands. The liver X receptors (LXRs) are an important pair of mammalian NRs that regulate lipid metabolism upon binding to cholesterol metabolites called oxysterols. A recent report that LXR activity is also regulated by binding to glucose (Mitro et al., 2007) expands the potential role of LXR in metabolic sensing and gene regulation. However, the hydrophilic nature of glucose and its low affinity for LXR present a challenge to central dogma about the nature of the NR-ligand interaction.

Nuclear receptors (NR) are an important family of transcription factors that play a major role in the regulation of cellular metabolism (Chawla et al., 2001). First characterized about 20 years ago, the founding members of the NR family are receptors for steroid and thyroid hormones that are proven targets for important pharmaceutical drugs. The human genome contains a total of 48 NRs, the majority of which do not bind to these classical endocrine hormones. Over the past two decades, natural as well as synthetic ligands have been identified for additional NRs, including related receptors for biologically active metabolites of the fat-soluble vitamins A and D. A remarkable range of naturally occurring molecules have been shown to bind and regulate "orphan" NRs (Table 1). These discoveries have provoked debate as to the criteria a molecule should fulfill to be anointed as a physiological ligand. A case in point is the liver X receptor (LXR), an important regulator of lipid metabolism whose activity is controlled by cholesterol metabolites called oxysterols (see Zelcer and Tontonoz, 2006 for a general review). A recent report from Mitro et al. (2007) has complicated this simple picture by suggesting that LXR may function as a sensor for glucose in addition to sterol metabolites.

There are two LXR subtypes, one that is predominantly expressed in the liver (LXRα, also called RLD-1; NR1H3) and one that is widely expressed (LXRβ, also called UR; NR1H2). LXRα was first described as an orphan receptor; soon thereafter, its activity was shown to be regulated by retinoids, which bind to its heterodimer partner RXR (Willy et al., 1995). Subsequently, oxysterols were found to activate LXR (Janowski et al., 1996), and biochemical and crystallographic studies prove that they do so by binding directly to the typical hydrophobic pocket in the C-terminal domain (Williams et al., 2003). The role of oxysterols as physiological ligands fits with their lipophilic nature, as well as with the phenotype of mice lacking LXRα, whose livers become filled with cholesterol when fed a Western diet